

# Novel steady state of a microtubule assembly in a confined geometry

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## Abstract

We study the steady state of an assembly of microtubules in a confined volume, analogous to the situation inside a cell where the cell boundary forms a natural barrier to growth. We show that the dynamical equations for growing and shrinking microtubules predict the existence of two steady states, with either exponentially decaying or exponentially increasing distribution of microtubule lengths. We identify the regimes in parameter space corresponding to these steady states. In the latter case, the apparent catastrophe frequency near the boundary was found to be significantly larger than that in the interior. Both the exponential distribution of lengths and the increase in the catastrophe frequency near the cell margin is in excellent agreement with recent experimental observations.

Microtubules are long, rigid polymers which play an important role in several cellular processes. They are highly dynamic structures, which are perpetually in a state of growth or shrinkage, and switch stochastically between these states. This behavior is called dynamic instability[1, 2]. The basic monomer unit of a microtubule is a dimer of  $\alpha$  and  $\beta$  tubulin, which is approximately 8 nm in length. The  $\alpha - \beta$  dimers are arranged head-to-tail along a microtubule in protofilaments (usually 13 per microtubule).

The highly dynamic nature of microtubules originates from the hydrolysis of  $\beta$ -tubulin bound GTP. Following hydrolysis, the GTP is converted to GDP [3], and the GDP-bound tubulin does not polymerize as well as its GTP counterpart. When the advancing hydrolysis front reaches the growing end of a microtubule, it starts de-polymerizing and the protofilaments start peeling off, releasing the GDP-tubulin into solution. Outside a microtubule, reverse hydrolysis takes place and polymerization events start all over again. Thus the microtubule constantly switches between phases of growth and shrinkage. The stochastic transition from growth to shrinkage is called *catastrophe* and the reverse transition is called *rescue*. The relative rates of catastrophe and rescue, combined with the velocities of growth and shrinkage determine the character of a given population of microtubules[4, 5, 6].

The first theoretical model of microtubule dynamics based on the dynamical instability mechanism was developed by Dogterom and Leibler[7]. In this model, microtubules are assumed to nucleate and grow from a flat substrate, and the dynamics is characterized by the velocity of growth ( $v_g$ ) and shrinkage ( $v_s$ ), and the frequencies of catastrophe ( $\nu_c$ ) and rescue ( $\nu_R$ ). In the absence of any boundary which restricts the growth, a steady state was achieved when  $\nu_R p_g < \nu_C p_s$ , characterized by an exponentially decaying distribution of lengths. When this condition was not satisfied, no steady state was reached, and the length distribution was Gaussian, with mean length increasing linearly with time, and the width evolving diffusively.

Inside cells, the microtubule growth is constrained by the presence of the cell boundary. Experimental observations have shown that the parameters of dynamics show a strong dependence on the proximity to cell boundary[8]. In particular, the catastrophe frequency was markedly higher near the periphery, compared to the cell interior. The obvious explanation for this difference was that the growing microtubule loses its ‘GTP cap’ upon hitting the cell boundary and is transformed to a shrinking state. In addition, the length distribution of microtubules was found to be exponentially increasing, with a possible dip near the

boundary.

In this brief communication, we show that the exponentially increasing length distribution of microtubules can be understood from the Dogterom-Leibler equations, and is a new steady state which is a direct consequence of the presence of the cell boundary. We compute the steady state distribution exactly, and find excellent agreement with experimental observations. We also show that the observed increase in the apparent catastrophe frequency near the cell margin can be understood quantitatively within this model.

Let us consider a set of microtubules nucleating from a substrate, and growing by the addition of tubulin dimers in the direction perpendicular to the plane of the substrate (the  $z$  axis). For simplicity, we ignore the three-dimensional structure of individual microtubules, and treat them as one-dimensional polymers. Nucleation is assumed to take place at empty nucleation sites at a rate  $\nu$ . A microtubule in growing state adds T-tubulin at a rate  $p_g$  per unit time, and a microtubule in shrinking state loses tubulin at a rate  $p_s$  per unit time. Also, a microtubule in growing state switches to shrinking state at a rate  $\nu_c$  (catastrophe frequency), and a microtubule in shrinking state switches to growing state at a rate  $\nu_R$  (rescue frequency). Both rescue and catastrophe are assumed to be purely stochastic events.

Our principal aim in this paper is to study explicitly the steady state of the system in the presence of a boundary. We assume that this boundary is located at  $z = l^*$ . We denote by  $p_+(l, t)$  the fraction of sites in the substrate which has a microtubule of length  $l$  at time  $t$  in growing state, and  $p_-(l, t)$  denote the same fraction in shrinking state. By convention, the fraction of vacant sites in the lattice at time  $t$  is denoted  $p_-(0, t)$ , and  $p_+(0, t) = 0$  at all times  $t$ . The discrete equations for the dynamics of this assembly, including growth, shrinkage, catastrophe and rescue events are given by

$$\frac{\partial p_-(0, t)}{\partial t} = -\nu p_-(0, t) + p_s p_-(1, t) \quad ; l = 0 \quad (1)$$

and

$$p_+(0, t) = 0$$

$$\frac{\partial p_+(1, t)}{\partial t} = \nu p_-(0, t) - p_g p_+(1, t) - \nu_c p_+(1, t) + \nu_R p_-(1, t) \quad ; l = 1 \quad (2)$$

$$\frac{\partial p_+(l, t)}{\partial t} = p_g [p_+(l-1, t) - p_+(l, t)] + \nu_R p_-(l, t) - \nu_c p_+(l, t) \quad ; 1 < l < l^* \quad (3)$$

$$\frac{\partial p_-(l, t)}{\partial t} = p_s[p_+(l+1, t) - p_-(l, t) + \nu_c p_+(l, t) - \nu_R p_-(l, t)] \quad ; 1 \leq l < l^* \quad (4)$$

The presence of the boundary affects the dynamics of the system in the following way: When a growing microtubule reaches a length  $l^*$ , it is instantaneously transformed to the shrinking state with length  $l^*$ . The equations representing this process are give by,

$$\frac{\partial p_-(l^*, t)}{\partial t} = p_g p_+(l^* - 1, t) - p_s p_-(l^*, t) \quad (5)$$

$$p_+(l^*, t) = 0$$

To find the steady state of the system, let us put all time derivatives to zero. From Eq.1 and Eq.2 we get the follwing relations.

$$p_-(0) = \frac{p_s}{\nu} p_-(1) \quad (6)$$

$$p_+(1) = \frac{\nu p_-(0) + \nu_R p_-(1)}{p_g + \nu_c} \quad (7)$$

After combining Eq.6 and Eq.7, we find that

$$p_-(1) = \frac{p_g + \nu_c}{p_s + \nu_R} p_+(1), \quad (8)$$

and, after using Eq.6 again,

$$p_-(0) = \frac{p_s}{\nu} \frac{p_g + \nu_c}{p_s + \nu_R} p_+(1) \quad (9)$$

For  $l > 1$ , we find the following relation between  $p_+(l)$  and  $p_-(l)$  from Eq.3 and Eq.4 (using only  $l > 1$  in Eq.4).

$$p_-(l+1) - p_-(l) = \frac{p_g}{p_s} [p_+(l) - p_+(l-1)] \quad l \geq 2$$

The general solution of this equation is

$$p_-(l) = \frac{p_g}{p_s} p_+(l-1) + C \quad l \geq 2 \quad (10)$$

where  $C$  is an unknown constant. After substituting Eq.10 in Eq., we obtain the following equation for  $p_+(l)$ .

$$p_g[p_+(l-1) - p_+(l) + \nu_R \frac{p_g}{p_s} p_+(l-1)]C = \nu_c p_+(l) \quad l \geq 2 \quad (11)$$

A trial solution to this equation has the form

$$p_+(l) = Aa^l + B \quad ; l \geq 1 \quad (12)$$

We now substitute this solution into Eq.11, and after equating terms with the same power of  $l$ , we obtain the following expressions for the constants  $a$  and  $B$ .

$$a = \frac{1 + \frac{\nu_R}{p_s}}{1 + \frac{\nu_c}{p_g}} \quad (13)$$

$$B = \frac{\nu_R p_s}{\nu_c p_s - \nu_R p_g} C \quad (14)$$

The constant  $C$  may now be determined as follows: From Eq.9, for  $l = 1$ , we have

$$p_s[p_-(2) - p_-(1) = \nu_R p_-(1) + \nu_C p_+(1), \quad (15)$$

whereas from Eq.10 we have another relation:

$$p_-(2) = \frac{p_g}{p_s} p_+(1) + C \quad (16)$$

We now substitute Eq.8 and Eq.16 into Eq.15 and solve for  $C$ , which gives  $C = 0$ . From Eq.14, this also implies  $B = 0$ . It remains to determine the constant  $A$ , which is found using normalization:

$$\sum_{l=0}^{l^*} p_-(l) + \sum_{l=1}^{l^*-1} p_+(l) = 1 \quad (17)$$

which may be written as

$$p_-(0) + p_-(1) + (1 + \frac{p_g}{p_s}) \sum_{l=1}^{l^*-1} p_+(l) = 1 \quad (18)$$

after using Eq.10. We now use Eq.8, Eq.9 and Eq.12 in Eq.18. The final result is

$$A = \left[ \frac{p_g}{\nu} + \frac{p_g}{p_s + (\frac{p_g + p_s}{p_s}) [\frac{a^{l^*} - a}{a - 1}]} \right]^{-1} \quad (19)$$

The solution in Eq.12 can also be written as

$$p_+(l) = Ae^{\alpha l} \quad ; \alpha = \log \left[ \frac{1 + \frac{\nu_R}{p_s}}{1 + \frac{\nu_C}{p_g}} \right] \quad (20)$$

The complete length distribution may now be written explicitly:

$$p(l=1) = A \left( \frac{p_g}{p_s} + a \right) \quad (21)$$

$$p(l) = A \left( 1 + \frac{p_g}{ap_s} \right) e^{\alpha l} \quad ; 1 < l < l^* \quad (22)$$

$$p(l=l^*) = \frac{p_g}{p_s} \frac{A}{a} e^{\alpha l^*}$$

If  $\frac{\nu_R}{p_s} < \frac{\nu_C}{p_g}$ ,  $\alpha < 0$  and we have an exponentially decaying solution. On the other hand, if  $\frac{\nu_R}{p_s} > \frac{\nu_C}{p_g}$ ,  $a > 1$  and  $\alpha > 0$ , and we have an exponentially increasing steady state distribution of lengths.

It is interesting to look at the behavior of the solution in the limit  $l^* \rightarrow \infty$ . From Eq.19, we see that, in this limit, a steady state is possible only if  $a < 1$ . For, if  $a > 1$ , then  $A \sim a^{-l^*}$  for large  $l^*$  and vanishes as  $l^* \rightarrow \infty$ . For  $a < 1$  and  $l^* \rightarrow \infty$ ,  $\frac{a^{l^*}-a}{a-1} \rightarrow \frac{a}{1-a}$ , and so

$$A_{l^* \rightarrow \infty} = \left[ \frac{p_g}{\nu} + \frac{p_g}{p_s + \left( \frac{p_g + p_s}{p_s} \right) \frac{a}{1-a}} \right]^{-1} \quad ; a < 1 \quad (23)$$

The exponentially decaying steady state length distribution when  $l^* = \infty$ , with  $\frac{\nu_R}{p_s} < \frac{\nu_C}{p_g}$ , has been predicted by Dogterom and Leibler in an earlier work[7]. The novel feature in the finite  $l^*$  case is the steady state with exponentially increasing distribution of lengths when  $\frac{\nu_R}{p_s} > \frac{\nu_C}{p_g}$ .

The exponentially increasing distribution of microtubule lengths has indeed been observed in experiments with real cells. Direct observation of microtubules inside cells has been made possible recently[8]. These experiments, done on centrosome-containing cytoplasts, observed almost persistent growth of microtubules almost up to the cell boundary. However, the catastrophe rate showed a dramatic increase within a zone about  $3\mu\text{m}$  near the cell margin ( $0.08\text{s}^{-1}$ , compared to  $0.005\text{ s}^{-1}$  in the cell interior). The other parameters describing the microtubule dynamics were  $\nu_R \approx 0.12\text{s}^{-1}$ ,  $v_g \approx 17.8 \pm 13.8\mu\text{m}/\text{min}$ ,  $v_s \approx 28.8 \pm 14.1\mu\text{m}/\text{min}$ . The parameters  $p_g$  and  $p_s$  are related to  $v_g$  and  $v_s$  as  $p_g = \frac{v_g}{\delta}$  and  $p_s = \frac{v_s}{\delta}$ , where  $\delta$  is the unit of length for our effective one-dimensional polymers. Since a microtubule consists of 13 protofilaments, and the length of a single tubulin dimer is 8 nm, this length scale is  $\delta = 8\text{nm}/13 \approx 0.6\text{nm}$ .

As a first test of our model, we compute the increase in catastrophe frequency near the cell margin. Let us consider all microtubules with length between  $l_1$  and  $l^*$ . The total number of such microtubules is given by  $N = \sum_{l_1}^{l^*} p(l)$ . Using the expression for  $p(l)$  from Eq.22, we find that

$$N = \frac{A}{\alpha} \left[ 1 + \frac{p_g}{ap_s} \right] [e^{\alpha l^*} - e^{\alpha l_1}]$$

The number of microtubules in this set undergoing catastrophe per unit time is given by

$$N^* = \nu_c N + p_g p_+(l^* - 1),$$

where the first term is the standard catastrophe term, and the second term represents the additional catastrophe events arising from the microtubules hitting the boundary. The apparent catastrophe frequency is given by  $\nu_c^* = \frac{N^*}{N}$ . After substituting for  $p(l)$  and  $p_+(l^* - 1)$ , we find that

$$\nu_c^* = \nu_c + \frac{p_g \alpha}{1 + \frac{p_g}{ap_s}} \frac{e^{-\alpha}}{1 - e^{-\alpha \Delta}} \quad (24)$$

where  $\Delta = \frac{l^* - l_1}{\delta}$ . After substituting for all the numerical values and for  $l^* - l_1 \simeq 3\mu\text{m}$  as in experiments, we find that  $\nu_c^* \simeq 0.0964\text{s}^{-1}$ . This is in excellent agreement with the experimentally measured value of  $0.08\text{ s}^{-1}$ .

It is also interesting to compare the experimentally measured value of  $\alpha$  with the theoretical value. The observed steady state length distribution was found to fit well with an exponential function  $P(l) \sim e^{\gamma l}$  with  $\gamma^{-1} \simeq 5.8\mu\text{m}$ [8]. We can convert this value to dimensionless units by multiplying with our unit of length, which gives  $\alpha_{exp} = \delta \gamma^{-1} \simeq 1.03 \times 10^{-4}$ . The theoretical value is found from Eq.20, using the measured values of all the parameters, and turns out to be  $\alpha \approx 1.5 \times 10^{-4}$ . This is also in very good agreement with the experimental value. The discrepancy between the computed and observed values may be attributed to the significant experimental error in the measurements of  $v_g$  and  $v_s$ .

To conclude, we have studied the steady state of a microtubule assembly in a confined geometry, where the growth of individual microtubules is restricted in length. We found that, in addition to the exponentially decaying length distribution in an infinite system, there is a novel steady state with exponentially increasing distribution of lengths. This prediction is in excellent agreement with experimental observations in real cells, and is thus a direct verification of the dynamical instability model of microtubule dynamics.

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